REPRODUCTIVE CYCLE IN CYPRINA ISLANDICA

VICTOR L. LOOSANOFF

U. S. Fish and Wildlife Service, Milford, Conn.

Cyprina (Arctica) *islandica* is a clam closely resembling in general appearance the more common species, *Venus mercenaria*. Externally it differs from the latter by having a shaggy, black periostracum which covers the shell. Formerly it was considered to be exclusively a European species but later it was found to be widely distributed on both sides of the North Atlantic. In Europe it ranges from the coasts of England and Norway to the Arctic, while on our Atlantic Coast it is found as far south as Cape Hatteras. However, since it is a cold water species, it is not very abundant south of New England.

A review of the literature shows that many aspects of the biology of *Cyprina islandica* have never been studied. According to Jørgensen (1946) practically nothing is known about the reproduction of this species and, therefore, virtually no literature exists on the subject. Lack of knowledge of the biology of *C. islandica* of our Atlantic Coast is probably due to the difficulty of collecting these clams because they live comparatively far from shore and in deep water. Moreover, since until recently they were not caught commercially, the specimens could not be readily obtained from fishermen.

The situation was changed in 1943 when, in an effort to increase war-time production of sea food, an extensive fishery of *C. islandica* was developed in the waters of Rhode Island and Massachusetts. Since then, samples of these clams have been more easily obtainable. As the fishery expanded and the clams began to appear as a canned product, and in fresh condition, the U. S. Food and Drug Administration in 1944 officially accepted "ocean quahog" as the common name for *C. islandica* (Rhode Island Report, 1945). In this article, for the sake of brevity, we shall call them clams.

Since the beginning of the war-time intensive fishery, new beds of the clams have been discovered. As a rule, these beds are located at a depth ranging from 70 to 120 feet, and are usually confined to either sand and mud or sticky mud (Arcisz and Sandholzer, 1947). Samples of the clams for our studies were collected from such beds located off Point Judith, Rhode Island in approximately 100–120 feet of water. They were collected often enough to record any significant changes in the conditions of the gonads. The clams constituting the samples were adults, several years old, and averaged 3½ to 4 inches in length.

The lack of instruments prevented us from recording the temperature of the water directly over the beds at the time the samples were collected. Fortunately, information on the seasonal temperature changes in that general area is available from the data of Merriman and Warfel (1948), who recorded the bottom temperatures at monthly intervals between September, 1943 and May, 1946 in the area near Point Judith. Because of the proximity of the localities where these temperatures were recorded to our clam beds and because of the strong tidal movements in that

general area, it is thought that there should be no pronounced local temperature differences.

Merriman and Warfel (1948) also gave data for bottom salinity of the same general region for the same period. Generally, the salinity was quite steadily maintained between 31.0 and 32.8 p.p.t., suggesting that this is an optimum salinity range for the existence and propagation of *C. islandica*.

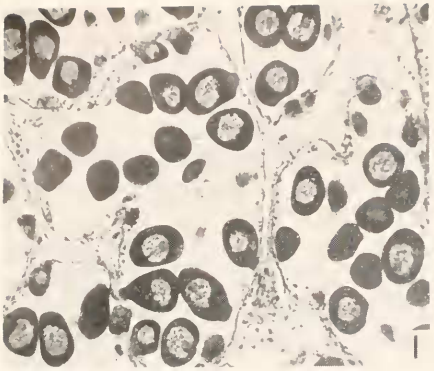
In studying the seasonal gonadal changes of *C. islandica* two approaches were used. The first consisted in histological studies of the material, which led to accurate determination of the condition of the gonads of each individual. Later on this information was used for the second, the statistical, approach, which gave us a quantitative conception of the successive changes in the gonad development during the year and of the progress of spawning during the season. The histological material will be discussed first, while the quantitative data and their analyses will be presented later and summarized in Tables I and II and Figure 13. Figure 13 was made the last figure of the article because of the convenience of not disturbing the continuity of the photomicrographs constituting Figures 1–12 inclusive.

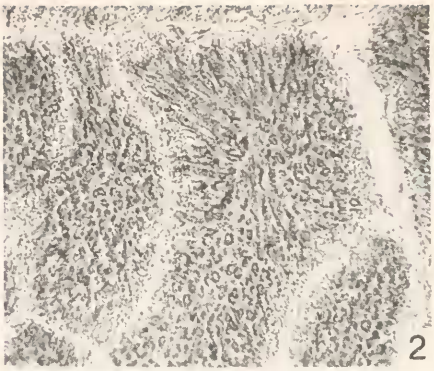
We may begin by considering the condition of the gonads of *C. islandica* just before the beginning of spawning. During the period of our studies this condition was reached by the end of June or early in July, when the temperature was near 13.0° C. Ripe female gonads were characterized at that time by extended follicles containing almost exclusively large, ripe ovocytes (Fig. 1). The walls of the follicles were touching each other, thus leaving virtually no space for the vesicular connective tissue. In males ripe spermatozoa predominated, occupying the largest portion of the follicular spaces, while cells of early stages of spermatogenesis were few in number and were confined to the area near the follicular walls (Fig. 2).

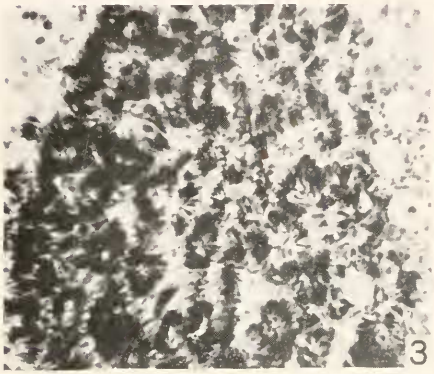
Even a superficial study of the gonads of ripe males often showed masses of "packed" spermatozoa, a condition caused by the arrangement of spermatozoa in the follicles in such a way that their heads were "packed" together creating the impression of a dense band (Fig. 3). A similar phenomenon was observed in *V. mercenaria* (Loosanoff, 1937a, 1937b) and several other lamellibranchs.

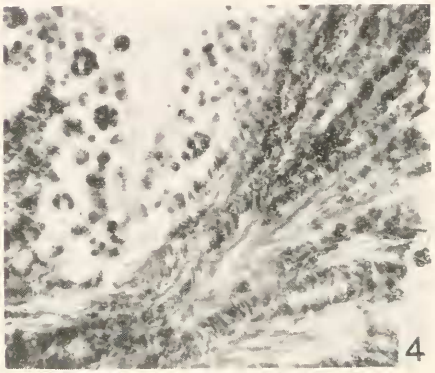
In addition to normal sex cells the follicles of many males also contained numerous multi-celled spherical dark bodies which were usually closer to the periphery of the follicles than to their centers (Fig. 4). Detailed examination of these bodies showed that they were cells of atypical spermatogenesis, noticed by Loosanoff (1937a) in *Venus mercenaria* and described by Coe and Turner (1938) in *Mya arenaria*. These cells apparently develop from the same type of spermatogonia as the normal ones but behave in a strikingly different way, undergoing several nuclear divisions, while still surrounded by the original mass of cytoplasm contained within the same cell membrane. Such abnormal bodies may contain from two to 16 nuclei (Fig. 5). It appears, as was noticed by Coe and Turner (1938), that in some cases such groups of nuclei may break apart and continue further individual development finally reaching the stage of spermatozoa. More often, however, they become pycnotic and are quickly cytolyzed.

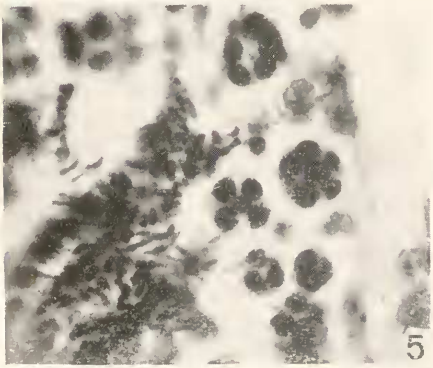
As is usual with lamellibranchs (Nelson, 1928a; Loosanoff, 1937b; Loosanoff, 1942), the entire population of *C. islandica* does not reach ripeness at the same time. We estimate that in the case of these clams only approximately 75 per cent were











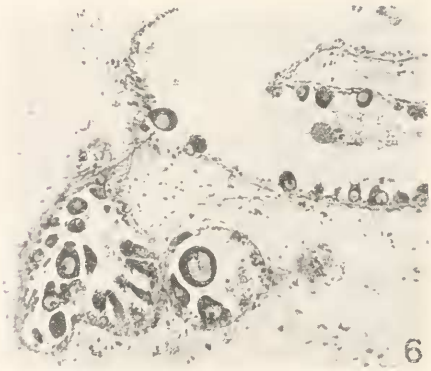


FIGURE 1. Gonad of ripe female. × 112.

FIGURE 2. Gonad of ripe male containing masses of spermatozoa ready to be discharged. × 112.

FIGURE 3. Section of male gonad showing "packed" spermatozoa. × 475.

FIGURE 4. Cells of atypical spermatogenesis occupying the periphery of a follicle containing ripe spermatozoa. × 475.

FIGURE 5. Different groups of cells (spherical) of atypical spermatogenesis. Normal spermatozoa are also present. × 1100.

FIGURE 6. Gonad of female in advanced stages of spawning. × 112.

TABLE I

Frequencies of occurrence of clams in different stages of sexual development at different dates between March 22 and November 1. Stage A: some ripe cells but generally unripe; Stage B: ripe but had not begun to spawn; Stage C: partly spawned; Stage D: completely spawned

Stages	Dates															
	3–22	4–5	4–19	5–3	5–17	5–31	6–14	6–28	7–12	7–26	8–9	8–23	9–6	9–20	10–4	10–18	11–1
A	10	7	8		3	4		5		0	0	0	0	0	0	0	0
B	3	2	2		1	3		13		5	4	8	6	1	0	0	0
C	0	0	0		0	0		2		3	4	10	12	6	3	8	1
D	0	0	0		0	0		0		0	0	1	3	6	4	9	5
Total	13	9	10		4	7		20		8	8	19	21	13	7	17	6

ripe or nearly ripe at the beginning of the spawning season. In the others the gonads still contained a large number of unripe cells. These conditions were especially noticeable in retarded males, the gonads of which contained predominantly the cells of early stages of spermatogenesis, although a few sperm were already present in the centers of some of the follicles.

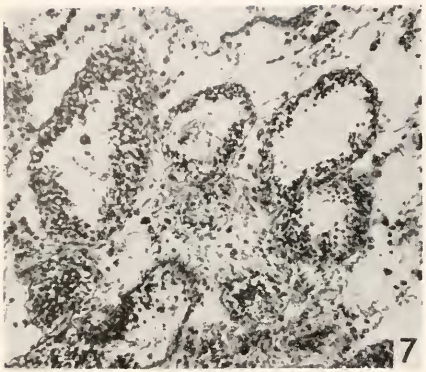
Judging by the condition of the gonads of both sexes, spawning began late in June or early in July when the water temperature over the beds was about 13.5° C. This temperature closely approached that reported by Nelson (1928b) for two other deep-water lamellibranchs, Astarte and Venericardium, which spawn near 12.0° C. Towards the end of July between 30 and 40 per cent of the clams were partly spawned but, as a rule, the quantity of spawn discharged remained small even then, thus indicating that a heavier and more general spawning was yet to follow. This was true because during August spawning continued, involving larger groups of the clam population, and toward the end of this month individuals of both sexes in advanced spawning conditions were becoming more common. In the females constituting this group the gonadal follicles were either empty or contained a few undischarged eggs (Fig. 6). In the males, most of the content of the follicles was discharged but the abnormal cells were still retained in large numbers, being confined to the periphery of the follicles where they were found among the normal cells

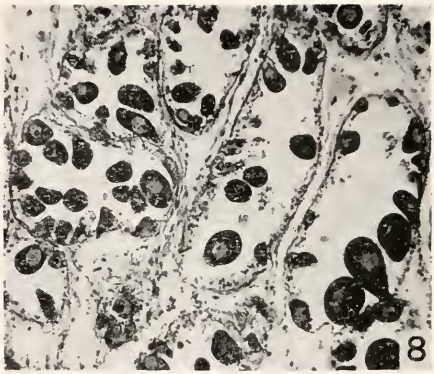
TABLE II

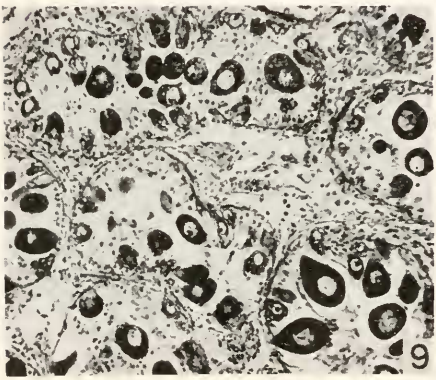
Cumulative percentage of clams at different stages of sexual development between March 22 and November 1. Stages as defined in Table I

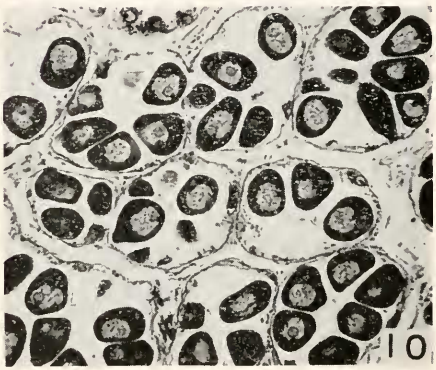
Stages	Dates																
	3–22	4–5	4–19	5–3	5–17	5–31	6–14	6–28	7–12	7–26	8–9	8–23	9–6	9–20	10–4	10–18	11–1
B + C + D	23.1	22.2	20.0		25.0	42.9		75.0		100	100	100	100	100	100	100	100
C + D	0	0	0		0	0		10.0		37.5	50.0	57.9	71.4	92.3	100	100	100
D	0	0	0		0	0		0		0	0	5.3	14.3	46.2	57.1	52.9	83.3
B + C + D (Adjusted*)	0	0	0		0	20.0		68.8		100	100	100	100	100	100	100	100

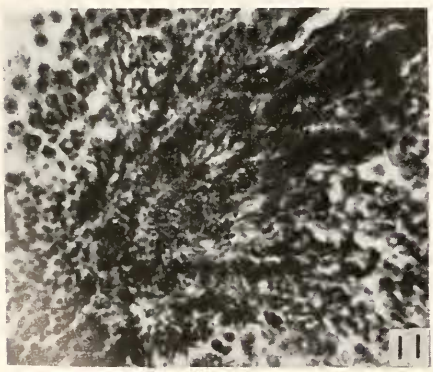
* Per cent in Stages B + C + D after subtraction of 20 per cent of sample size from Stage B and from total.











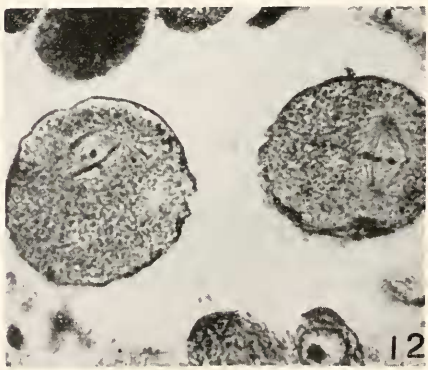


FIGURE 7. Almost empty follicles of male in advanced stages of spawning. × 112.

FIGURE 8. Female gonad in late October still containing some old ovocytes but also forming new ones to be discharged next year. × 112.

FIGURE 9. Female gonad in December showing largely small growing ovocytes, but also containing some that are morphologically ripe. × 112.

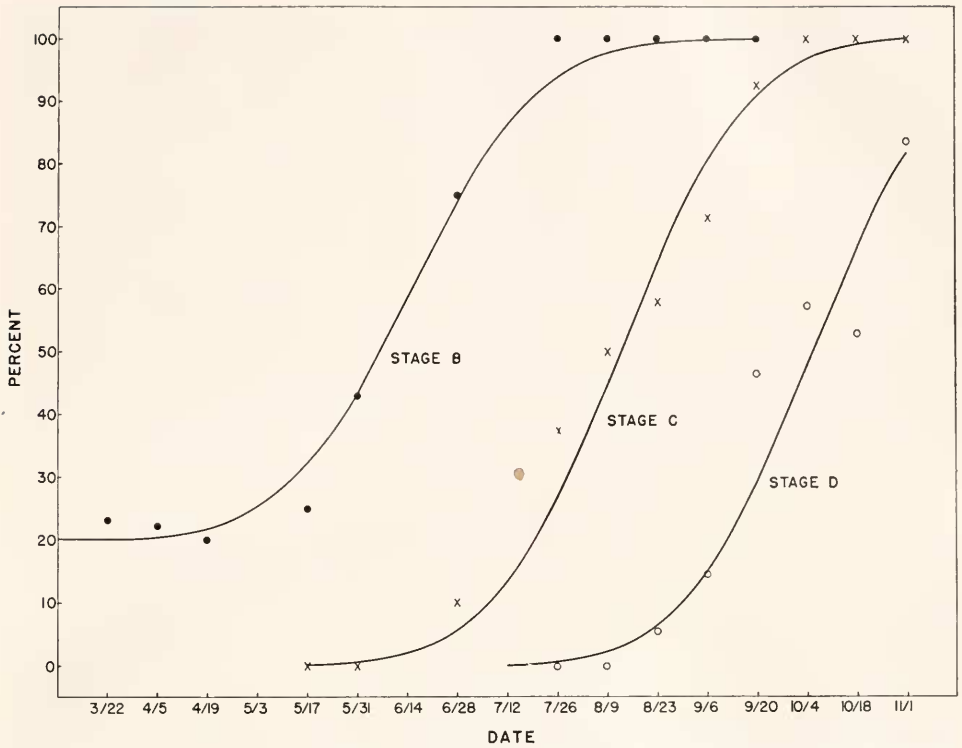


FIGURE 13. Percentage of clams in successive stages of sexual maturity at different times of the year. Stage B: ripe but had not begun to spawn; Stage C: partly spawned; Stage D: completely spawned. Additional explanation in text.

in earlier stages of development (Fig. 7). The emptied follicles were not contracted but remained distended, as was noticed for *V. mercenaria* (Loosanoff, 1937b), and in some instances they gradually became filled with vacuolated cells.

During September, when the water temperature was at its maximum of about 15.0° C., and when partially and fully spawned clams were becoming progressively more numerous, ripe males, showing the so-called "packing" arrangement of the sperm, and ripe but unspawned females still were encountered. In October the temperature of the water was slowly declining but, nevertheless, spawning continued and during the first part of the month was, perhaps, even more active than in September. By the middle of the month the majority of the animals had completed spawning, and towards the end of the month the number of clams with spawn was rapidly diminishing (Tables I and II, Fig. 13).

FIGURE 10. Morphologically ripe gonad of a female during late December or in January. × 112.

FIGURE 11. Male gonad in late December or in January already containing ripe spermatozoa. × 475.

FIGURE 12. Eggs ready to be discharged in spawning showing dissolution of the germinal vesicle and the formation of chromosomal spindle. × 475.

During the last part of October and the early part of November the clams passed through the period of recovery consisting in part of resorption of unspawned material. However, as in *V. mercenaria*, there appeared to be no true indifferent period, when the follicles are free of all cells except the indifferent ones as is the case of *C. virginica* (Loosanoff, 1942; Coe, 1943). On the contrary, even then the sex of the clams was well defined, a condition which was especially clearly visible in the females because of the presence in their gonads of large numbers of ovogonia and young ovocytes. Sometimes such ovocytes began to develop before the old ones were discharged (Fig. 8). The presence in the follicles of well defined sex cells during all periods of the annual gonadal cycle indicated, of course, that sex in adult *C. islandica* is quite stable and that sex reversal, as was recently shown for *V. mercenaria* (Loosanoff and Miller, 1950), rarely, if ever, occurs.

After passing through the period of resorption of unspawned material, the clams enter a period of extremely rapid gametogenic activities which continues into December, regardless of the rapidly declining temperature. Some females possessed follicles containing either well grown ovocytes (Fig. 9) or ovocytes that were already so developed that they appeared morphologically ripe (Fig. 10). In males also all stages of development, from relatively unripe males in early stages of spermatogenesis to those the follicles of which contained many spermatozoa, were found (Fig. 11). If some of the material was taken from the spermaries of such advanced males and placed in sea water, the spermatozoa would soon begin swimming in their typical circular motion, indicating the ripeness of the gonads.

During the middle of winter, when the temperature is at its lowest, sometimes approaching only 1.5° C., gametogenesis was slowed down but probably not entirely arrested. Even at such a low temperature the clams remain somewhat active because in a laboratory experiment, in which they were placed in water of about 1.0° C., the clams opened the shells, extended their siphons, and pumped water, thus indicating that they were not hibernating.

With a gradual increase of the temperature in the spring, gametogenesis became progressively more rapid and eventually, by the end of June or early July, a state of physiological and morphological ripeness was achieved by individual clams.

Although the observations on the seasonal gonadal changes of the clams were carried on throughout the entire year, only those data obtained between March 15 and November 1 were analyzed statistically. The late fall and winter conditions were not included in the quantitative analysis. During that period, as already pointed out in the histological discussion, the gonads went through the recovery stage followed by rapid gametogenesis as the result of which approximately 20 per cent of the clams appeared to be morphologically ripe by about March 15.

For the quantitative studies the data were grouped in intervals of two weeks, accepting the mid-point of each of these intervals as the date for all the observations of that group. There were seventeen such intervals but in three of them observations were lacking (Table I).

All clams were assigned to one of four stages depending upon gonad condition. Stage A contained clams which were generally unripe but which, nevertheless, contained some ripe cells. The clams of Stage B were ripe, at least morphologically, but had not begun to spawn. Stage C consisted of partly spawned clams, while Stage D contained those that were completely spawned.

The observed frequencies of the clams in each of the four stages are presented in Table I. Inspection showed that the percentage of clams which have entered Stages B, C or D increases as the season progresses (Table II, Fig. 13). The analysis was based on the provisional assumption that the relation underlying this increasing percentage is the cumulative normal curve, the curves for the different stages of maturity differing only in the dates of their succession. In general, Figure 13 is a comparison between the cumulative percentages obtained from Table II and the theoretical cumulative normal curves which have been postulated.

Since it was found that approximately 20 per cent of the clams possessed gonads that appeared to be morphologically ripe by March 15, the lower limit of the percentage of clams that had entered Stage B at that date was not equal to zero but to about 20 per cent. Our hypothesis was extended to allow for this percentage, only assuming the normal cumulative curve of ripening for the remaining 80 per cent of the population. Accordingly, the number of clams closest to 20 per cent of the total sample in each group was subtracted from Stage B and from the total, giving the values of adjusted percentage in Stages B + C + D (Table II). No adjustment was made in the analysis of Stages C + D and D.

The cumulative percentages given in Table II, adjusted in the case of the class B + C + D, were used to estimate the approximate dates when 50 per cent of the clam population would be expected to have entered Stages B, C or D. Analysis showed that these dates probably fall within ± 3 days of June 15, August 13 and October 6 respectively. Thus, the time interval between the date when 50 per cent of the clams are expected to enter Stage B (ripe but unspawned) and Stage C (partly spawned) was 59 days, and between Stage C and Stage D (completely spawned), 54 days. It should be emphasized once more, however, that the mean date of June 15 applies only to the ripening of 50 per cent of those clams that were not morphologically ripe by March 15. As is evident in Figure 13, the mean date for the population as a whole is about June 4.

The same analysis showed a population standard deviation of 29 days. This means, for example, that 95 per cent of the population would enter a given stage during a period extending from about 60 days before to 60 days after the mean date.

As mentioned above, the standard error of the three critical dates was estimated to be ± 3 days. Larger samples of clams should provide greater precision of these estimates, but at present such refinement does not seem necessary. It was also possible to estimate the goodness of fit of the cumulative normal model, making a separate test of the assumption of equal standard deviations for the three stages of development. After a maximum likelihood fitting by the methods of probit analysis the residual $\chi^2 = 8.68$. We have allowed 28 degrees of freedom in the original 4×14 table, disregarding those classes with an expected frequency of less than one clam. In fitting the curves given in Figure 13, 18 constants have been used: 14 sample sizes, 3 means and the standard deviation. This leaves 10 degrees of freedom for χ^2, showing a satisfactory agreement. If individual slopes are fitted for the three cumulative curves, the sum of the 3 residual χ^2's = 6.77 with 8 degrees of freedom. The difference, $\chi^2 = 1.91$ with two degrees of freedom, arises from lack of parallelness of the three lines, and is not significant.

Our attempts to induce spawning in ripe *C. islandica* always failed. Methods, such as rapid increase in temperature, addition of suspension of sex products,

changes of pH, salinity, etc., produced no results. During the spawning experiments it was found that the clams do not tolerate well temperatures over 27.0° and that at temperatures of 30.0° C. or higher they looked as if anesthetized, a condition manifested by relaxation of the adductor muscles and by expansion of the foot. Usually, subjection to such a high temperature was followed within a few days by a heavy, usually complete, mortality.

Natural unprovoked spawning was observed in only two cases. Once it occurred in the middle of winter, on February 3, 1945, when a single female kept in an aquarium in a cold room, the temperature of which was increased by direct sun to only 9.0° C., discharged a large number of eggs. The actual act of spawning was not seen but the eggs were found in a mass lying next to the clam. The eggs ranged from approximately 80 to 95 μ in diameter.

The second spawning occurred on July 19, 1948 when clams of both sexes, used several days prior to that date in an unsuccessful spawning experiment, spawned during the night. The temperature at the time of spawning was 22.0° C. The size of the eggs varied between 85 and 90 μ. Some of the eggs were fertilized and showed normal early development.

Attempts to fertilize artificially the eggs stripped from ripe females always failed because such eggs, even after being placed in sea water, still retained intact their germinal vesicles. Under normal conditions the germinal vesicle dissolves while the egg is still in the gonads of the mother clam, just before being discharged in the process of spawning. Immediately upon dissolution of the germinal vesicle a chromosomal spindle is formed (Fig. 12). After that the egg is ready for fertilization. In the stripped eggs, however, such changes do not occur and, therefore, fertilization is impossible. In this way *C. islandica* again closely resembles *V. mercenaria* in which artificial fertilization also cannot be achieved.

In preparing this article I feel especially obliged to my colleague, David W. Calhoun, for his statistical analysis of the data. I also wish to extend my sincere thanks to Charles A. Nomejko for the preparation of the photomicrographs and to William Arcisz for collecting and shipping to me many samples of clam gonads on which this article is based.

Summary

1. The main period of gametogenesis in both sexes of *Cyprina islandica* occurs in late fall and early winter. In December or January the gonads of some clams present a morphologically ripe appearance, such individuals constituting approximately 20 per cent by March 15. Gametogenesis is slowed down but not entirely arrested during late winter and is resumed at a rapid rate with the spring increase in temperature.

2. Spawning begins near the end of June or early in July when the water temperature is approximately 13.5° C.

3. The dates when 50 per cent of the clam population should be expected to enter the stage of being ripe but not having begun to spawn, the stage of partial spawning, and the stage of completed spawning should fall within ± 3 days of June 4, August 13 and October 6 respectively.

4. In adult *C. islandica* the sexes are separate and well defined even immediately after spawning, thus indicating that sex reversal is uncommon.

5. The eggs of *C. islandica* cannot be artificially fertilized.

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